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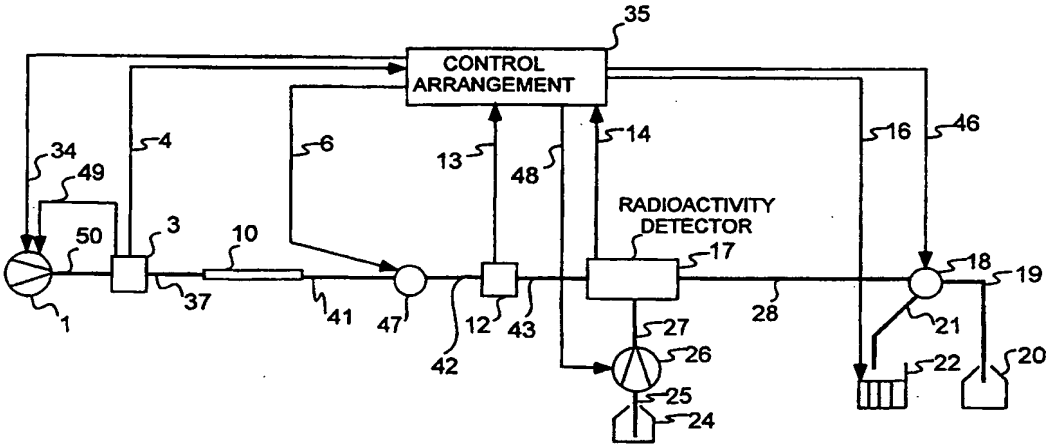
(54) Title: APPARATUS AND METHOD FOR RADIOACTIVITY MEASUREMENT IN LIQUID CHROMATOGRAPHY



(57) Abstract

A stop-flow method and apparatus for measuring radioactivity in a liquid chromatographic eluate in which flow is stopped and resumed so that radioactivity of desired portions of the eluate can be measured accurately in a stationary manner. The sample is passed through a chromatographic column (10) to a radioactivity detector (17) via valve (47) under the control of controller (35). The valve (47) is closed in response to signals from a timer, the radioactivity detector (17) or other detector (12) to stop eluate flow and permit stationary measurement. In a second embodiment, eluate fractions may be stored in a capillary storage device prior to passage to the radioactivity detector (17) for stationary measurement.

APPARATUS AND METHOD FOR RADIOACTIVITY MEASUREMENT IN LIQUID CHROMATOGRAPHY

BACKGROUND OF THE INVENTION

This invention generally relates to an apparatus and method for measurement of radioactivity in liquid chromatography.

It is well known in the prior art that for accurate quantitation of radioactivity in a liquid sample, one has to measure or count the sample in a liquid scintillation counter for a period of time. The lower the radioactivity, the longer the measurement or counting time in order to achieve certain counting accuracy. This is due to the nature of radiation, which statistically follows the Poisson distribution. Due to the fact that emission from radioactive isotopes is a random process, the accuracy in detection of those isotopes based on the detection of emission depends greatly on the measurement time for the samples. The longer the samples are measured, the more accurate results will be obtained. The percent error based on the 95% confidence interval can be calculated as follows:

Percent error (or %2sigma)=200/(square root of total counts accumulated)

Therefore, counting for a longer period of time is an effective way and in many cases is the only way to achieve the lower percent error or more accurate results, even though higher counting efficiency and lower counting background also contribute to higher counting accuracy or sensitivity.

Therefore, the best approach to solve this problem in the prior art is to first collect fractions of the eluate into scintillation vials using a fraction collector, then mix each eluate fraction with a volume of scintillation cocktail, and finally measure the radioactivity of those fractions in a stationary manner in a liquid scintillation counter (LSC). Since each fraction can be measured in a stationary manner for a longer period of time, the required counting accuracy can be achieved for each eluate fraction. The results from those fractions then are used to reconstruct the radio-chromatogram. The disadvantages associated with this method are obvious:

(a) Involving intensive manual operations and increasing the cost per analysis which results in poor productivity.

(b) Generating a great amount of radioactive solid and liquid wastes including scintillation vials, contaminated scintillation cocktail and solvents.

(c) Increasing the potential of creating unsafe working environments.

Eliminating this fraction collection/LSC operation in modern laboratories would improve not only the productivity but also working environments related to workers and to the public in general due to the benefit of waste reduction. Consequently, a need exists for new techniques in radioactivity measurement in liquid chromatography, which not only would have greater accuracy and sensitivity of radioactivity measurement for all desired portions of chromatogram, but also are operated in an automated and environment friendly manner.

SUMMARY OF THE INVENTION

The present invention provides a stop-flow radioactivity measurement apparatus and method designed to satisfy the aforementioned needs. The stop-flow radioactivity measurement is carried out by a stop-flow system that stops and resumes

(a) collecting fractions of an eluate from liquid chromatography in a storage device and

(b) feeding the fractions individually back into the radioactivity detector for counting in a stationary manner.

Again, the operation as to when and where to collect fractions can be activated by either a timed sequence or other signals such as radioactivity signal observed in the radioactivity detector.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the first embodiment of the stop-flow radioactivity counting apparatus pursuant to the present invention;

FIG. 2 shows the second embodiment of the stop-flow radioactivity counting apparatus of the present invention;

FIG. 3 shows the detailed sequences for a typical stop-flow radioactivity counting process of the present invention;

FIG. 4 shows the detailed sequences for another mode of operation for counting only the peaks in the present invention.

14, with sample injector 3 through signal line 4, valve 47 through signal line 6, with pump 26 through signal line 48, with detector 12 through signal line 13, valve 18 through signal line 46, and fraction collector 22 through signal line 16.

Valve 47 is preferred in order to stop the flow of mobile phase completely to achieve the best performance in separation under stop-flow operation. Valve 47 can be a on/off valve or switching valve which enables the flow at one position and disables or block the flow of mobile phase(s) at another position, which is preferably disposed immediately after column 10. However, valve 47 can also be disposed immediately after radioactivity detector 17 if radioactivity detector 17 and other devices between radioactivity detector 17 and column 10 can tolerate the pressure during the stop-flow operation.

Pump 26 is used for pumping either scintillation fluid or non-radioactive solvent(s) or a solvent mixture in container 24 through line 25 and to radioactivity detector 17 through line 27 for either liquid scintillation counting purpose or flushing out the residual radioactivity residing inside the flow cell of radioactivity detector 17. No scintillation fluid is needed for a solid cell.

Sample injector 3, disposed between pump 1 and column 10 via lines 50 and 37, is either a manual injector (such as a loop on a multiple port switching valve) or automatic injector (auto-sampler). Detector 12, such as a UV(ultraviolet) detector, can be connected on-line between valve 47 and radioactivity detector 17 through lines 42 and 43. The connection lines between the column 10 and valve 47 is 41.

different time intervals or fractions for counting radioactivity by radioactivity detector **17** in a stationary manner. As an example, the first five fractions (**F1** through **F5**) are shown in FIG. **3B**. Fraction **F1** contains the eluate collected in the flow cell (either a solid or a liquid cell) of radioactivity detector **17** between time points **T0** and **T1**. Fraction **F2** is the eluate collected from time points **T1** through **T2**, etc.

When a sample is injected onto sample injector **3**, a signal is generated and sent through signal line **4** to control arrangement **35** which triggered pump **1**. Alternatively, the injection signal can be sent directly from sample injector **3** to pump **1** through signal line **49** for triggering of the run. Once pump **1** is triggered, the solvent elution or gradient will start. The sample is pushed onto column **10** for fractionation and the eluate is collected in the flow cell (not shown) of radioactivity detector **17**. If a liquid cell is used, pump **26** also starts pumping liquid scintillation fluid which is mixed with eluate from line **43** before the liquid cell. If a solid cell is used, pump **26** does not pump anything into radioactivity detector **17**. When time point **T1** is encountered, control arrangement **35** will send a stop-flow signal to pump **1** to stop the flow of the mobile phase through signal line **34**, and both flow and gradient are stopped or paused. At the same time, if valve **47** is used, control arrangement **35** also sends a signal through line **6** to turn valve **47** to the close or off position in order to stop the flow of mobile phase completely. Once the flow is stopped, radioactivity detector **17** starts measuring (or counting) the radioactivity contained in the flow cell (from time points **T1** to **a)**). The radioactivity is measured by detecting the flash of photons generated by the interaction of radiation particles (such as beta particles from carbon-14 isotopes, etc.) with either liquid or solid scintillator by using usually a pair of

At time point **b**), the flow and gradient (if any), of pump 1 is resumed for another cycle. If a solid cell is used, pump **26** stops pumping any solvent. As we can see, this stop-flow radioactivity counting method cycle includes the following steps: collecting the eluate into the flow cell; stopping the flow and stopping (pausing) the gradient (if any); counting the radioactivity inside the flow cell in a stationary manner; removing the residual radioactivity from the flow cell; and finally resuming the flow and gradient (if any) of pump **1** and pump **26** (if a liquid cell is used). When the gradient is resumed, it starts from the point where it was stopped previously. This will ensures the best performance in separation and resolution comparable to the prior art continuous run or analysis.

After finishing the stop-flow counting cycle for fraction **F1**, next cycle continues for next fraction **F2**, etc. In FIG. **3**, we observed five counting cycles (from time points **T0** through **n)**), each of which counts the corresponding fraction (**F1** through **F5**). The results of the counting cycles are shown in the radio-chromatogram in FIG. **3E**, which has five data points, each of which representing the counting results from corresponding fractions (**F1** through **F5**). In this example, a peak is accurately detected in fraction **F4** at time point **T4** of FIG. **3E**. The time scale on FIG. **3A** is the time scale for a continuous run. The time scales in FIG. **3B** through FIG. **3D** are the actual time scales during the stop-flow counting process. The time scale in FIG. **3E** is the reconstructed time scale by eliminating the time spent during the stop-flow counting cycles. The reconstructed radio-chromatogram represents the radioactivity distribution of fractions in FIG. **3A**. There was not changes in the chromatographic separation and retention times using the stop-flow counting method comparing to the normal continuous run in the prior art. One explanation is

scintillation fluid to radioactivity detector **17** if a liquid cell is used. If a solid cell is used, pump **26** does not pump anything. When radioactivity detector **17** detects **Peak 1** (based on either a higher radioactivity than the preset threshold or other algorithms such as changes in slope, etc.), a fraction of eluate, the size of which is determined either by the predefined time intervals or levels of radioactivity detected or detection of the end of the peak. After a fraction of eluate is collected, the control arrangement will stop the flow of pump 1 and the gradient (if any) and count the radioactivity inside the flow cell for a predefined period of time or to accumulate enough counts to reach certain levels of counting accuracy.

In this example, fraction **F6**, containing eluate from time points **T0'** through **T1'**, is counted first (from time points **T1'** to **p**)). The accumulated counting data are calculated as the mean which represents the radioactivity level of fraction **F6**, which is shown at time point **x**) in FIG. **4E**. After the counting of fraction **F6** is finished, the content of the flow cell is flushed out by solvent(s) delivered by pump **26** between time points **p**) and **q**). At time point **q**) the flow and gradient (if any) of pump **1** is resumed until time point **r**), where fraction **F7** (eluate from time points **T1'** to **T2'**) is ready for counting.

Fraction **F7** is subjected to a similar counting cycle as shown for fraction **F6** from time points **r**) to **t**), resulting the data at time point **y**) calculated as the mean. When no peak is detected, the flow of pump **1** continues until the next peak (**Peak 2**) is encountered. In this example, **Peak 2** is narrow enough so that only one fraction (fraction **F8**) containing eluate from time points **T3'** through **T4'** is needed to count the entire peak. The entire **Peak 2** is counted in a stationary manner in the counting and flushing cycle between time points

Depending on the width of the regions of interest, each region can be counted as more than one fraction.

As shown in FIG. 1, fraction collector 22 and waste container 20 can be used to collect useful radioactive components detected by radioactivity detector 17. Valve 18 is a three-way valve which is controlled by control arrangement 35 through signal line 46. Fraction collector 22 is controlled also by control arrangement 35 through signal line 16.

The components of interest in the eluate can be collected using fraction collector 22. The fraction collection can have several modes of operation. The first one is to collect peak(s). When a peak is detected in radioactivity detector 17 based on predefined criteria, the eluate is directed to the fraction collector through the valve after a delay time which compensates the time needed for eluate traveling from the outlet of radioactivity detector 17 to valve 18. When the end of the peak is detected, the eluate is directed to the waste container through valve 18 after the delay time. The second mode is collection of predefined regions of interest. Again, when the beginning of region(s) of interest is encountered, the control arrangement will send signals through line 46 which turns the valve toward the fraction collector. When the end of the regions of interest is encountered, the valve is turned back to the waste container by control arrangement 35 through signal line 46 for collection of waste. This is an excellent tool to collect the needed peaks or components for further analysis by other means such as mass spectrometry or NMR (nuclear magnetic resonance). This method is also good for waste management where separation of high level of radioactive wastes from low level or non-radioactive wastes are desired, thus saving cost in waste disposal.

through line **45**. Valves **2** and **44** is connected through line **8**. Valve **2** is connected with control arrangement **35** through signal line **5**. Storage devices **31** and **33** are connected in series and connected to valve **2** and **44** through lines **23**, **32** and **7**.

The stop-flow radioactivity counting method using embodiment of FIG. **2** is shown as follows. Since storage devices **31** and **33** can store the fractions of the eluate into the capillary tubing, the eluate can be collected and stored in the storage devices and counted after the LC run is finished. The size and length of the capillary tubing on storage devices **31** and **33** can vary depending on the fraction size and total number of fractions per LC run.

At default, valve **2** is at the position connecting lines **36** and **37** and valve **44** connecting lines **28** and **23**. In the other words, the eluate flows through the following lines and devices sequentially: **1**, **50**, **3**, **36**, **2**, **37**, **9**, **40**, **10**, **41**, **42**, **12** (if any), **43**, **17**, **28**, **23**, **33**, **32**, **31**, **7**, **8**, **45**, **18**, **19**, and finally arrives waste container **20**.

Once a sample is injected into the LC system through sample injector **3**, a sample injection signal is generated and sent through line **4** to the control arrangement. Control arrangement **35** then sends a signal through line **34** to trigger pump **1** to start the run. The control arrangement **35** will turn storage device **33** to the first position to collect eluate which fills the first capillary tubing through signal line **29**. After the predefined time interval for collection of the first fraction, the control arrangement **35** will turn the storage device **33** into its second position to collect the second fraction, etc. When all the capillary tubings on storage device **33** are filled with eluate fractions, the control arrangement **35** will turn

equals to the volume of the fraction in case of a liquid cell or the combined volume of the fraction and the liquid scintillation fluid in case of a liquid cell, this flushing step can be eliminated since the following fraction will replace the previous fraction inside the flow cell. This is the counting cycle for counting one fraction stored in storage device **33**.

Similarly, control arrangement **35** will send a signal to pump **1** to resume the flow of the mobile phase and repeat the cycle described above to count the radioactivity of the subsequent fractions one by one. After all the fractions are counted, a radio-chromatogram containing all the counting results, each of the data points representing the counting results of each fraction, can be obtained.

The desired radioactive peaks or regions of interest can be collected into fraction collector **22** which is controlled by control arrangement **35** through line **16**. When peak(s) is detected in radioactivity detector **17**, control arrangement **35** will turn valves **44** and **18** such way that the eluate will go through: **28**, **44**, **45**, **18**, **21** and finally to fraction collector **22**. If more than one peaks are being collected, the control arrangement will advance the fraction collector to the next tube or vial by sending a signal through line **16**. Same procedures can be used for collecting region(s) of interest. Moreover, more than one fraction can be collected from one region of interest.

It is possible to divide one fraction into several portions with same or different sizes and count them individually. This will result in better resolution on the radio-chromatogram. In this case, only a fraction of the whole fraction content stored in each individual capillary tubing will be fed into radioactivity detector **17** for counting in a stationary manner. Between each portion of whole fraction, no delay time is

fractions stored on storage devices **33** or **31**. The content of each capillary tubing can be counted once or divided into many portions and counted separately. After each counting process, the flow cell can be flushed with a organic solvent or solvent mixture delivered by pump **26**. This will ensure the elimination of the possible cross contamination resulting from the residual radioactivity from the previously counted sample.

The detailed description of the present invention as shown above will enable anybody with ordinary skills in the art to make and use the present invention without any undue experiments.

It is thought that the stop-flow measurement apparatus and method of the present invention and many of its attendant advantages will be understood from the foregoing description and it will be apparent that various changes may be made in the form, construction and arrangement of the parts thereof without departing from the spirit and scope of the invention or sacrificing all of its material advantages, the form hereinbefore described being merely a preferred or exemplary embodiment thereof.

REFERENCE NUMERALS IN DRAWINGS

No.	Part Name	No.	Part Name
1	pump	20	waste container
2	valve	21	line
3	sample injector	22	fraction collector
4	signal line	23	line
5	signal line	24	container
6	signal line	25	line
7	line	26	pump
8	line	27	line
9	valve	28	line
10	column	29	signal line
11	column	30	signal line
12	detector	31	storage device
13	signal line	32	line
14	signal line	33	storage device
15	line	34	signal line
16	signal line	35	control arrangement
17	radioactivity detector	36	line
18	valve	37	line
19	line	38	line

7. An apparatus according to claim 6, wherein said valve means is a on/off valve disposed immediately downstream of said column means.

8. An apparatus according to claim 6, wherein said valve means is a switching valve which enables the flow of said eluate at one position and disables or blocks the flow of said eluate at another position.

9. An apparatus according to claim 6, said valve means is disposed immediately downstream of said radioactivity detection means .

10. An apparatus according to claim 1, further comprising a fraction collector, disposed downstream of said radioactivity detection means, so that the portions of said eluate containing radioactive components are collectable.

11. An apparatus according to claim 1, wherein said radioactivity detection means comprises of a flow cell.

12. An apparatus according to claim 11, further comprising a pump means for delivering liquid scintillation fluid and flushing said flow cell.

13. An apparatus according to claim 1, further comprising a storage means for collecting fractions of said eluate into a plurality of capillary tubing connected onto said storage means.

14. An apparatus according to claim 13, wherein said storage means is a multiple position valve.

radioactivity detector,

whereby the radioactivity in said eluate will be measurable in a stationary manner for a period of time.

22. A method according to claim 21, wherein said collecting fractions of said eluate in a storage means is activated according to a timed sequence.

23. A method according to claim 21, wherein said collecting fractions of said eluate in a storage means is activated in response to radioactivity signal observed in said radioactivity detector.

Fig. 2

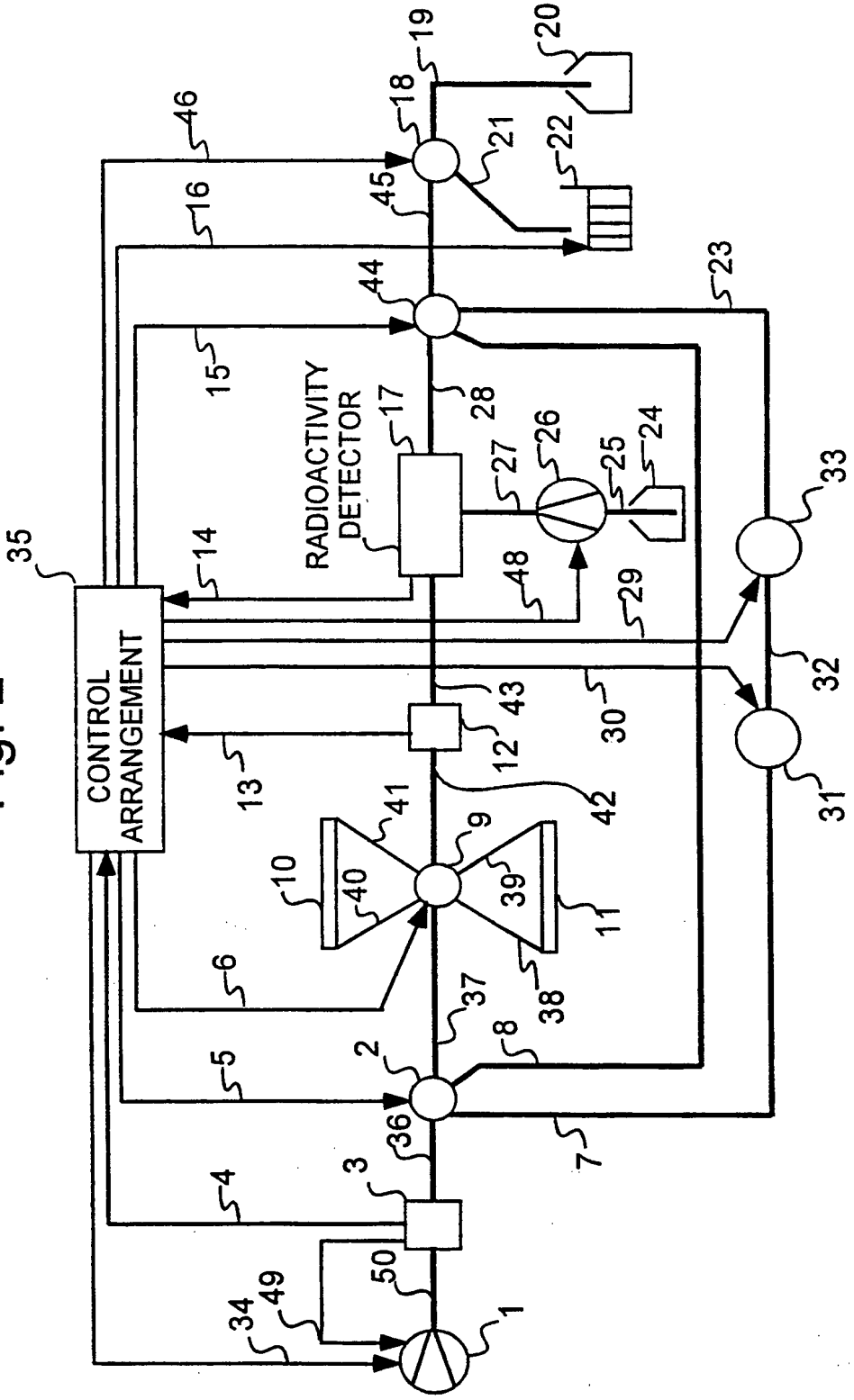
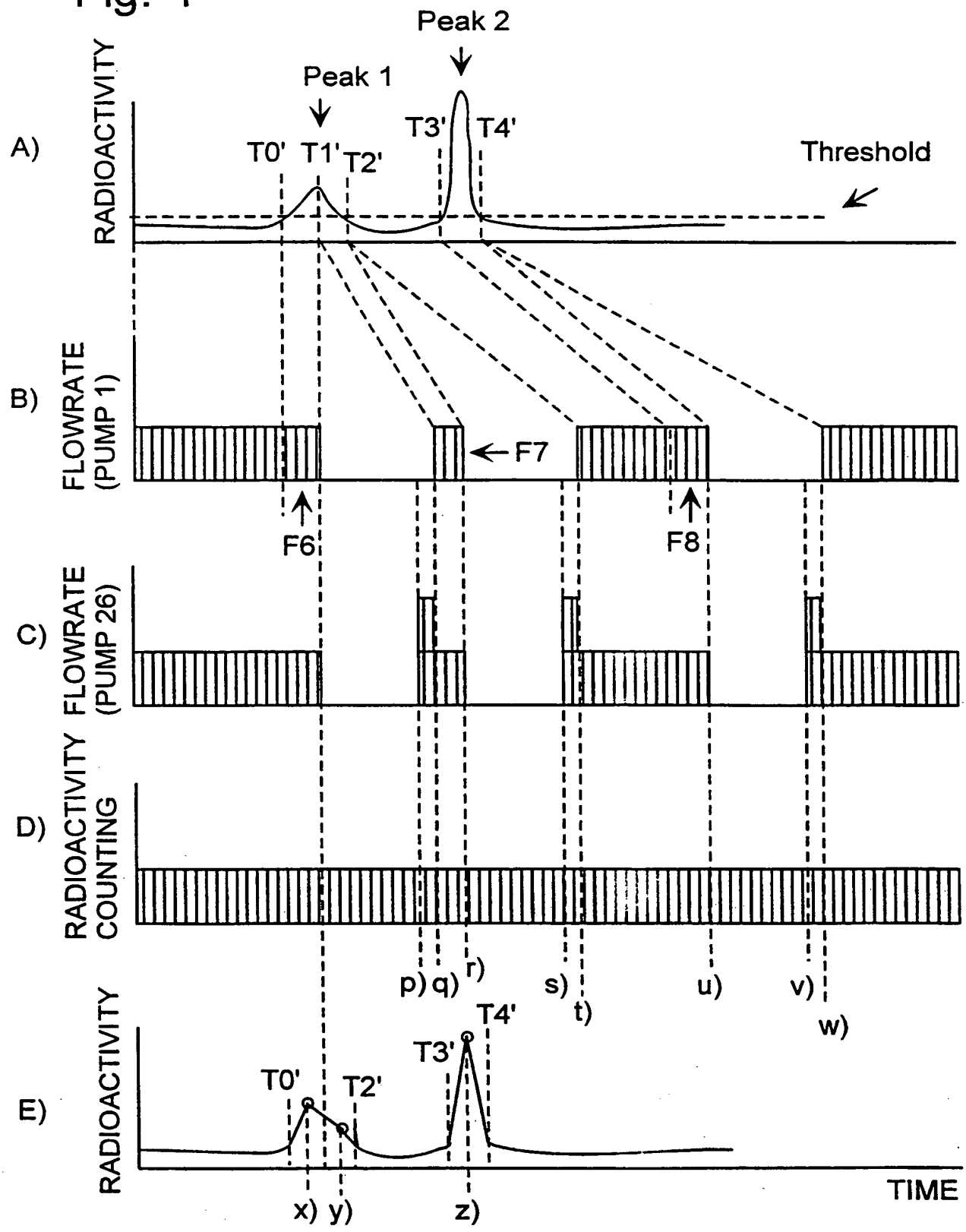


Fig. 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/20324

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

436/57, 161; 422/70, 71; 73/61.56, 61.58; 210/198.2, 656; 250/328, 364